Supporting information

Microfluidic Immunoassays for Sensitive and Simultaneous Detection of IgG/IgM/Antigen of SARS-CoV-2 within 15 min

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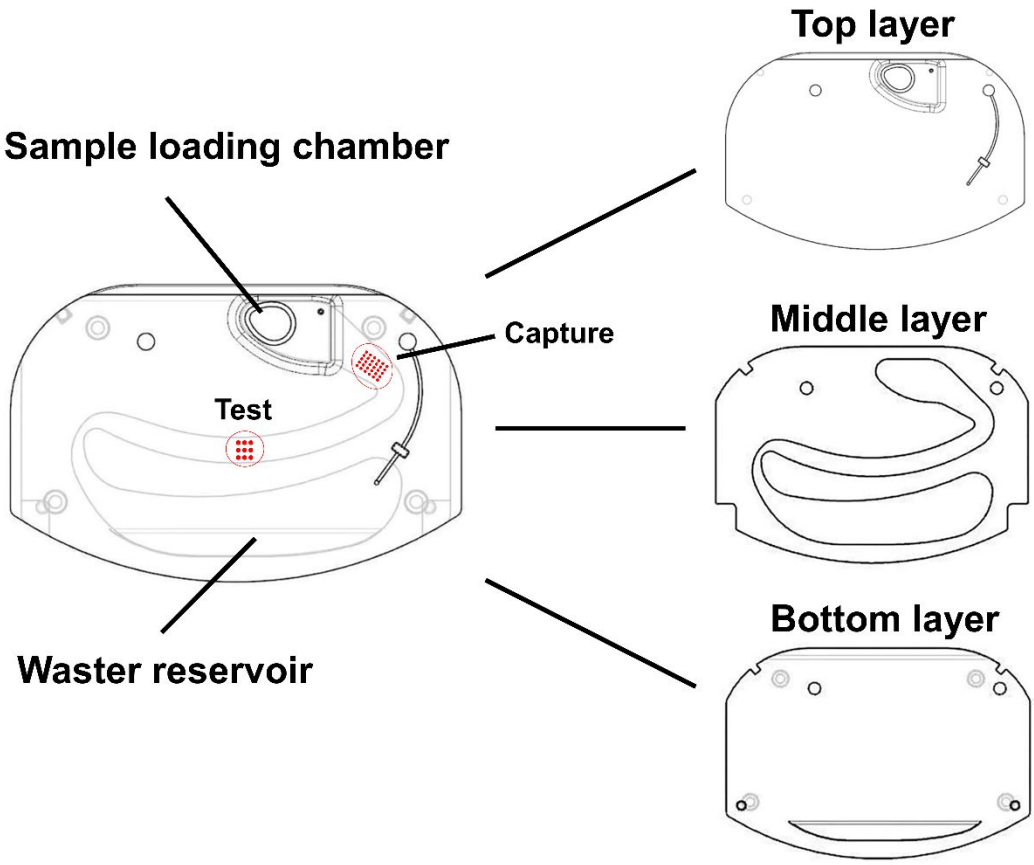

Figure S1. Scheme of microchip for fluorescence immunoassay. (Capture: FMS labeled mouse anti-human IgG/IgM antibody or SARS-CoV-2 capture antibody (rabbit anti-human polyclonal antibody) was patterned in order to capture analyte; Test: SARS-CoV-2 antigen or SARS-CoV-2 detection antibody (mouse anti-human monoclonal antibody) was patterned in order to capture FMS labeled complexes for fluorescence detection.)

Experimental

Materials

Mouse anti-human IgG/IgM antibody, SARS-CoV-2 antigen (recombinant antigen), SARS-CoV-2 capture antibody (rabbit anti-human polyclonal antibody) and SARS-CoV-2 detection antibody (mouse anti-human monoclonal antibody) and sample buffer (0.1M PBS, pH 7.4, 0.1% TX-100) were from Shanghai Suchuang Diagnostic Products Co., Ltd. (Shanghai, China). Fluoro-max dyed carboxylate-modified microparticles (fluorescent microspheres, 200 nm,) and sulfo-NHS were obtained from Thermo Fisher Scientific (China) Co., Ltd. (Shanghai, China). 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and bovine serum albumin (BSA) were purchased from Sigma Aldrich Trading Co., Ltd. (Shanghai, China). 2-(N-morpholino) ethanesulfonic acid sodium salt (MES sodium salt, 50 g) was obtained from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). Microchips were manufactured by Shanghai Suchuang Diagnostic Products Co., Ltd. (Shanghai, China).

A portable microchip instrument (Shanghai Suchuang Diagnostic Products Co., Ltd, consists of three parts: a microchip base bracket with centrifugation function, a laser-induced fluorescence detector, and a display. The microchip instrument enables to analyze three immunoassay microchips each time.

Clinical samples such as serum and pharyngeal swabs were collected and tested in Shanghai East Hospital (affiliated East Hospital of Tongji University, Shanghai, China). The written informed consent had been obtained since the start of the project. All

experiments on clinical samples were performed in compliance with the Medical Research Ethics Committee, Shanghai Pediatric Hospital. All of clinical samples were detected directly in this assay and each extra sample was stored at -20 °C for further study.

Conjugation of fluorescent microspheres with capture antibodies.

The conjugation of fluorescent microspheres (FMS) with capture antibody against IgG/IgM/Antigen was achieved according to the manufacturer's instructions with minor modification. FMS were coated with each type of capture antibody through amide bond under the activation of EDC and NHS.

Preparation of immunoassay microchip.

Detection antibody of each marker (IgG/IgM/Antigen) and antigen against SARS-CoV-2 were patterned on the surface of bottom substrate layer of a microchip by matrix nano-spotting strategy (Ad3220 Aspirate/Dispense Platform (BIODOT, USA)). 0.5 μL of 0.5 mg/mL SARS-CoV-2 antigen (recombinant antigen) (for IgG/IgM detection) or SARS-CoV-2 detection antibody (for antigen detection) was spotted onto the test region in a matrix of 3×3 spots (each spot ~ 50 nL); 3 μL of FMS coated mouse anti-human IgG/IgM capture antibody or SARS-CoV-2 capture antibody (rabbit anti-human polyclonal antibody) (1.08 mg/mL) was spotted onto capture region in a matrix of 5×6 spots (each spot ~ 100 nL). The patterned bottom layers were then dried at 37 °C for 4 h. Finally, the immunoassay microchips were assembled by the double-sided adhesive layer and stored at 2-30 °C for use.

Immunoassays on microchip

The fluorescence immunoassay detection of IgG/IgM/Antigen in the developed microchips was performed as follows. 10 μL of sample was directly added to sample loading chamber and followed by 70 μL of sample buffer**,** then the microchip targeting one of the analytes (IgG/IgM/Antigen) and incubated for 10 min. Three immunoassay chips were placed in the portable immunoassay microchip instrument in a single run and centrifuged for seconds to remove the residual liquid in the channels following fluorescence detection for 1 min. The fluorescence intensity of test (T value) can be automatically obtained from the instrument for sample analysis.